NTP
National Toxicology Program

Trimethylsilyldiazomethane (TMSD)

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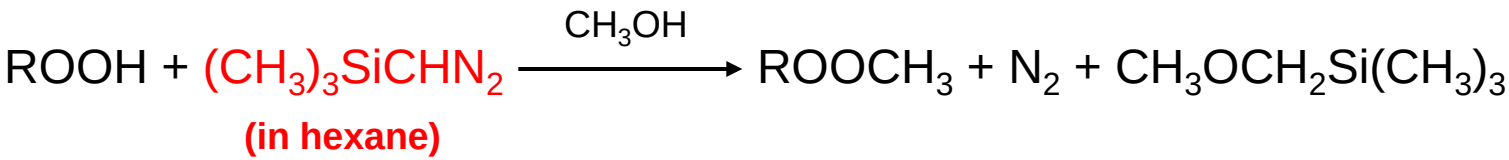
TMSD Background

- TMSD is a synthetic methylating reagent used by chemists for organic synthesis and in analytical methods.

- TMSD is commonly used for the derivatization of carboxylic acid groups into methyl esters. The methyl esters are easily identified by GC-MS and thus are ideal derivatives for the characterization of carboxylic acids.



- TMSD was originally developed as a less toxic substitute for the highly unstable (explosive) and toxic compound diazomethane (CH_2N_2).

Diazomethane Safety and Toxicity

- Diazomethane (CH_2N_2) is highly flammable and gas/air mixtures are explosive.
- Documented cases of severe pulmonary injury and death after inhalation exposure to diazomethane in humans.
- Lethal in acute animal inhalation toxicity studies.
- Diazomethane has been described as carcinogenic to the lung. The ACGIH has classified it as a suspected human carcinogen; however, the IARC has it listed as not classifiable.
- The OEL for diazomethane (TLV, PEL and REL) is 0.2 ppm (TWA).

TMSD Toxicity

- There is very little data regarding the toxicity of TMSD.
- TMSD has been reported to be a skin irritant, to be harmful if ingested, to target multiple organs and to be a suspected reproductive toxicant. These effects of TMSD have been reported in MSDS sheets provided by chemical suppliers; however, supporting data are not available.
- Exposure to TMSD in an occupational setting (chemical laboratory) is likely to occur via dermal contact or inhalation.
- Structure-Activity Relationships: no predictions (in regards to potential metabolites, protein binding, toxicity and carcinogenicity) can be made based on low structural similarity.
- An acute dermal toxicity study in rabbits concluded that TMSD is non-toxic via dermal exposure.
- No inhalation studies with TMSD have been performed to date.

TMSD Nomination

- OSHA nominated TMSD largely as a result of the deaths in 2008 and 2009 of two chemists who were exposed to TMSD in the workplace.
- Both deaths were a result of acute and progressive respiratory distress from pulmonary injury believed to be caused by exposure to TMSD.
- It is possible that TMSD itself, diazomethane and/or other chemical species generated through the breakdown of TMSD (stability issue) were responsible for the pulmonary effects.
- TMSD is marketed as a safe alternative to diazomethane; however, this may not be true.
- There is therefore a need to characterize the inhalation toxicity of TMSD and to establish its inhalation exposure limits.

Key Issues

- The health and safety of individuals performing stability and toxicity studies with TMSD as well as chemical containment. Conservative safety precautions will be taken to protect those individuals involved.
- The stability and breakdown of TMSD into diazomethane and/or other chemical species.
- The effect of the solvent carrier for TMSD on toxicity and in the generation of pure TMSD vapor.
- The feasibility of generating a specified atmospheric concentration of pure TMSD vapor in order to perform an acute inhalation toxicity study.
- Nose-only versus whole body exposure and breathing differences between rodents and humans.

Specific Aims

1. Characterize the availability, purity (solvents) and stability of TMSD.

2. Determine the feasibility of generating controlled atmospheric concentrations of pure (and stable) TMSD vapor and characterize the stability of TMSD in artificial lung fluid.

3. Perform acute inhalation toxicity study.

4. Perform additional *in vitro* and *in vivo* studies with TMSD.

- **Because there is limited information available for TMSD, specific aims 1 and 2 need to be addressed prior to performing an acute inhalation toxicity study (specific aim 3).**

Proposed Approach

Specific Aim 1

- Screen for bulk liquid purity and chemical stability of TMSD from different vendors. TMSD has been reported to be stable in neat or in hydrocarbon solution. Pure TMSD may not be commercially available since most applications use TMSD in hexane or diethyl ether solvent.
  - A preliminary search of suppliers has shown that TMSD is primarily available in liquid form: 2M solution (~10%) in methylene chloride, diethyl ether or hexane solvent.
  - Pure (neat) TMSD is not readily available as it is considered by vendors to be too dangerous for handling.

Proposed Approach

Specific Aim 2

- Perform study to investigate the feasibility of generating a controlled atmospheric concentration of pure TMSD vapor and to assess the stability of TMSD vapor.
  - TMSD may break down into diazomethane and/or other chemical species.
- The generation of pure TMSD vapors in the presence of a solvent carrier could be problematic. In addition, the effects of the carrier/vehicle on toxicity must be considered.
  - Diethyl ether has been reported to have low acute toxicity in humans and animals and is also used as an anesthetic.
- The stability of TMSD in artificial lung fluid will also be assessed.
  - TMSD may break down into diazomethane and/or other chemical species upon contact with liquid lining the lung surface.

Proposed Approach

Specific Aim 3

- Two week (dose range-finding) inhalation study will be performed using whole body exposure (5 days per week, 6 hr per day) to assess the acute lung toxicity of TMSD in rats and mice (male and female).
  - The respiratory tract and other major organs will be collected from all animals upon completion of the exposure regimen for histopathologic evaluation.

Proposed Approach

Specific Aim 4

- Additional studies may be conducted with TMSD to evaluate:
  - Inhalation toxicokinetics
  - Mechanisms of TMSD-induced pulmonary toxicity
  - Acute dermal toxicity (skin irritation and corrosion) *in vitro*
  - Eye irritation *in vitro*
- Because of its acute toxicity, a subchronic inhalation study with TMSD does not appear to be warranted.
  - Determine whether routine, low dose and long term exposure occurs with chemists in a laboratory setting.

Significance and Expected Outcome

- The data obtained will be used to:
  - Update MSDS (and other chemical reviews) for TMSD.
  - Establish an inhalation OEL for TMSD.
  - Provide risk assessment and regulation of TMSD.

- If TMSD is found to be unstable and readily forms diazomethane, then an acute inhalation toxicity study will likely not be necessary. Diazomethane has previously been shown to be highly toxic (and lethal) upon inhalation. An OEL for diazomethane is already established and can be used to provide further risk assessment and regulation of TMSD.

- Further evaluation will be needed if TMSD is unstable and breaks down to yield chemical species/toxic products other than diazomethane.